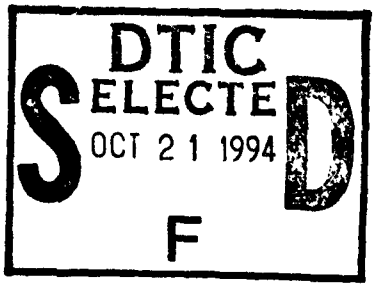

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RAPID AND SELECTIVE CYCLIC VOLTAMMETRIC MEASUREMENTS OF EPINEPHRINE AND NOREPINEPHRINE AS A METHOD TO MEASURE COSECRETION FROM SINGLE BOVINE ADRENAL MEDULLARY CELLS

by

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ABSTRACT

Background-subtracted cyclic voltammetry at a scan rate of 800 V/s with carbon fiber microelectrodes has been used to detect and differentiate between epinephrine and norepinephrine. At very positive potentials (>1 V vs. SSCE) in pH 7.4 aqueous buffer, a second oxidation wave is observed for epinephrine, a secondary amine. In contrast, the second oxidation wave is not observed for norepinephrine, a primary amine. The amplitude of the second wave for epinephrine is enhanced when the waveform employed does not allow reduction of the electrogenerated o-quinone back to epinephrine. This indicates that the oxidation process at the second wave must be preceded by adsorption of the o-quinone at the electrode surface. The temporal response of the method was investigated by iontophoretic ejection of catecholamine onto an electrode. The response time was found to be limited by the repetition rate of the cyclic voltammograms (16.7 ms in this work). This electrochemical technique was used to resolve catecholamine release from individual vesicles of cultured bovine adrenal medullary cells. Most of the adrenal medullary cells released either epinephrine or norepinephrine but 17% of the cells released mixtures of these two compounds. In these cells, each secretory vesicle appeared to contain either epinephrine or norepinephrine.

INTRODUCTION

Catecholamines are an important class of biological chemical messengers and their ease of oxidation provides a useful and rapid way to measure their release in biological systems with microelectrodes. Background-subtracted fast-scan cyclic voltammetry has been shown to be useful for catecholamine measurement in a quantitative and qualitative way both *in vitro* and *in vivo*[1,2]. In addition, this technique allows the temporal resolution necessary to time resolve secretion events at single cells. However, the catecholamines are similar in structure and difficult to differentiate electrochemically. Traditionally measurements of catecholamine secretion were obtained in systems where a single catecholamine existed. However, this is not the case for secretion from bovine adrenal medullary cells where both epinephrine and norepinephrine exist. One way to discriminate between these two molecules is to monitor the cyclization reaction of epinephrine which occurs after electrooxidation[3,4]. With this method, however, the fastest possible data collection rate that can be obtained is one cyclic voltammogram per second, the time scale of the rate of the epinephrine cyclization.

The only structural difference between norepinephrine and epinephrine is that the amine on the ethyl group of epinephrine is methylated whereas the amine of norepinephrine is not. In this work we use this difference to distinguish between these two compounds because secondary amines are more easily oxidized than primary amines[5]. The utility of this technique is demonstrated in secretion measurements from bovine adrenal medullary cells.

Adrenal medullary cells release epinephrine and norepinephrine in packets from individual secretory vesicles. Release of these individual packets can be temporally resolved with fast electrochemical measurements[6-9]. These cells average 8 μm in radius[10] and a 6 μm radius carbon fiber microelectrode can be placed adjacent to the cell to measure the release of electroactive components. Each cell contains about 30,000

vesicles[11] with an average radius of 150 nm[12]. When one of these vesicles fuses with the plasma membrane, the contents of the vesicle are released into the external medium in a process called exocytosis. The catecholamines are stored in these vesicles at a concentration of approximately 360 mM[13].

Prior work has demonstrated that release of catecholamines from the vesicular matrix occurs on a millisecond time scale[14]. Thus, it is important to accomplish the measurement of the catecholamines on this rapid time scale. In this work, this has been done with a scan rate of 800 V/s repeated at 16.7 ms intervals. The response to rapid concentration changes with this technique has also been confirmed.

EXPERIMENTAL SECTION

Electrochemistry

All electrodes except the 6 μm electrode used in the collector-generator experiment were constructed as previously described[15] using 5 μm radius carbon fibers (Thornell P-55, Amoco Corp. Greenville, SC). The tips were polished at a 45° angle on a diamond dust embedded micropipette beveling wheel (K.T. Brown Type, Sutter Instrument Co., Novato, CA). After polishing, there was about a 1 μm thickness of glass capillary surrounding the carbon fiber at the tip. The 3 μm radius electrode used in the collector-generator experiment was constructed from the same type of carbon fibers. The electrode tips were flame-etched and insulated with a polymer coating instead of glass before being polished as previously reported[16,17].

Electrochemical measurements were taken with an EI-400 potentiostat (Ensman Instrumentation, Bloomington, IN) in the two-electrode mode with respect to a locally constructed sodium saturated calomel reference electrode (SSCE). For experiments with iontophoresis, the potentiostat was used in the three-electrode mode with a chromel wire auxiliary electrode. Data obtained for the collector-generator experiment was digitized with a digital oscilloscope (Nicolet 310, Nicolet Instrument Corporation, Madison, WI) and transferred through the RS232 port to the computer. Electrodes were calibrated using a flow-injection apparatus.

For cyclic voltammetry, a triangle waveform was applied to the electrode every 16.7 ms at a scan rate of 800 V/s. The cyclic voltammetric response was filtered at 5 kHz and the amperometric response was filtered at 50 Hz with a two-pole filter. Timing between cyclic voltammograms and data digitization was with a Labmaster interface (DMA, Scientific Solutions, Solon, OH) and a preliminary version of software (CV6, Ensman Instrumentation, Bloomington, IN). Cyclic voltammograms were background-subtracted with responses collected just prior to introduction of catecholamine to the electrode surface. Current-time

traces of the catechol oxidation were taken by averaging the data collected in the 100 mV-range around the peak oxidation potential for this wave. Current-time traces of the second oxidation were taken by averaging the data between 1400 mV and 1425 mV and subtracting out the data between 1000 mV and 1200 mV to remove noise artifacts that occur at potentials greater than 1000 mV. Between 1000 mV and 1200 mV, no current due to catecholamine oxidation occurs so that this subtraction method does not remove any catecholamine signal from the data.

Iontophoresis

The constant current device used for iontophoresis was locally constructed and battery-powered to minimize line noise. The current supply was referenced to the low-impedance auxiliary electrode of the potentiostat because this was found to be the optimum method to prevent electrical "cross-talk" between the two systems. Attempts were made initially to reference the iontophoretic current source to the reference electrode of the potentiostat, but a significant amount of electrical "cross-talk", in the form of a current spike, appeared at the working electrode when the iontophoretic current source was triggered to change between the negative (holding) and positive (ejecting) currents. We found that the low-impedance auxiliary electrode was able to sink the iontophoretic current without affecting the electrochemical measurements and without causing a current spike at the carbon-fiber microelectrode when the current of the iontophoretic system was switched. To further reduce noise, the current supply was optically isolated at the trigger.

For iontophoresis, a micropipette with a 2 μm inner-diameter tip was placed 1 μm away from the carbon electrode. The micropipette was filled with 200 μM catecholamine in pH 7.4 tris/HCl buffer with 150 mM NaCl. The solution in the micropipette was connected to the constant current source with a chromel wire. Timing of the constant current application was computer controlled. A current of 200 nA was used to eject catecholamine from the micropipette into the bulk solution.

Cultured Cells

Single cell, iontophoresis and collector-generator measurements were taken on the stage of an inverting microscope (Axiovert 35, Zeiss, Eastern Microscope, Raleigh, NC). Electrodes were positioned with a piezoelectric micropositioner (Kopf Model 640, Tujunga, CA). A pressure ejection device (Picospritzer, General Valve Corp., Fairfield, NJ) applied pressure to a micropipette with a 10 μm inner-diameter tip.

Bovine adrenal glands were obtained from a local slaughterhouse and cells were isolated from the glands as previously described[18] using a Renograffin density gradient to obtain an epinephrine-rich fraction and a norepinephrine-rich fraction[19,20]. Both of these fractions were used in the experiments. Experiments were done at room temperature from one day to five days after isolation of the cells from the glands. Spacing distance between the single cell and the electrode was determined by bringing the electrode into contact with the cell until the cell was visually deformed. The electrode was then retracted 1 μm from the cell surface as measured with the piezoelectric micropositioner.

Reagents and Solutions

Flow-injection analysis and iontophoresis experiments were conducted in a 200 mM Tris/HCl (Sigma, St. Louis, MO) buffer solution at pH 7.4 with 150 mM NaCl. Epinephrine (free base), norepinephrine hydrochloride and 3,4-dihydroxybenzylamine hydrobromide, used for iontophoresis measurements and electrode calibration, were also obtained from Sigma. Solutions of the catecholamines were prepared by dilution of 50 mM stock solutions in 0.1 N $HClO_4$. Single cell experiments were conducted in pH 7.4 buffer with 50 mM Tris/HCl, 150 mM NaCl, 4.2 mM KCl, 11.2 mM glucose, 2 mM $CaCl_2$, 0.7 mM $MgCl_2$. Cells were stimulated by pressure ejection (10 psi for 3 seconds) of a solution containing 50 mM Tris/HCl buffer, 90 mM NaCl, 64.2 mM KCl, 11.2 mM glucose, 2 mM $CaCl_2$, 0.7 mM $MgCl_2$ at pH 7.4. HEPES buffer, as well as many other biological buffers that contain tertiary amines, is electroactive at the potential at which the second oxidation wave of epinephrine

is observed and thus could not be used. Solutions were prepared in distilled deionized water (Mega Pure System MP-3A Corning Glass Works, Corning, NY). The cell culture medium was Dulbecco's Modified Eagle's Medium/Ham's F12 Medium (Gibco Laboratories, Grand Island, NY). Collagenase (Type 1, Worthington Chemicals, Freehold, NJ) was used for digestion of glands. Renograffin-60 (Squibb Diagnostics, New Brunswick, NJ) was used to separate the cells into epinephrine-rich and norepinephrine-rich fractions.

RESULTS AND DISCUSSION

Optimum Waveform for Fast-Scan Cyclic Voltammetry of Catecholamines

Figure 1A and 1B show background-subtracted cyclic voltammograms of epinephrine and norepinephrine made by monitoring the oxidation of catecholamine to o-quinone and the reduction of o-quinone back to catecholamine. This is the way that background-subtracted cyclic voltammograms of catecholamines have traditionally been made. The peak oxidative currents and peak reductive currents occur at the same two potentials for both epinephrine and norepinephrine however, so that selectivity is not possible with this technique.

Figure 1C shows a cyclic voltammogram of epinephrine in which the potential limits of the waveform are -450 and +1425 mV. This cyclic voltammogram shows the oxidation of catecholamine to o-quinone at about +500 mV, a second oxidation wave at about +1400 mV and then the reduction of o-quinone to catecholamine at about -150 mV on the reverse scan. The epinephrine cyclic voltammogram can be distinguished from the norepinephrine cyclic voltammogram in figure 1D on the basis of the second wave. The second wave in figure 1C is quite small however and for lower concentrations of epinephrine, as might be seen at a single cell, it is difficult to measure. For this work, it is desirable to maximize the amplitude of the second wave to easily differentiate between the two catecholamines.

It was found that the amplitude of the second wave can be increased by changing the potential limits of the triangle waveform to +100 mV and +1425 mV. The cyclic voltammograms for the oxidation of the two catecholamines with this potential waveform are shown in figure 1E and 1F. While this waveform eliminates the o-quinone reduction wave, it was found to be optimum to distinguish between these two species at a fast scan rate. The postulated electrochemical reactions for these cyclic voltammograms are shown in scheme 1, where reaction (1) is the reversible oxidation of catecholamine to o-quinone and reaction (2) is the irreversible oxidation of amine to imine. This scheme explains why the second

wave is only possible for epinephrine.

Alternatively, the second oxidation wave could be the oxidation product formed from the o-quinone. However, this is less likely because there is no literature evidence to support this scheme.

In figure 1C, the second oxidation wave is small relative to the catechol oxidation wave presumably because the o-quinone must be adsorbed onto the surface of the electrode before the second oxidation can occur. Indeed, when the reductive wave is scanned less of the second wave is found. When the reductive wave is not scanned, as in figure 1E, the o-quinone stays near the carbon surface for a longer amount of time, allowing further oxidation. Additional data to support this hypothesis is presented later in this paper.

The wave observed at 1400 mV in figure 1E exhibits hysteresis and does not have a defined peak. The direction of the triangle wave scan is reversed in the middle of the second oxidation wave because the background current increases dramatically at the carbon electrode at more positive potentials. This large increase in the background current results in an accompanying loss in signal-to-noise ratio. As the applied potential is scanned in a negative direction, the oxidation continues in the high potential region. This leads to the hysteresis at high potentials when the cyclic voltammogram is then background subtracted.

Response Time

The technique of iontophoresis has been used to characterize electrode responses to rapid introduction of electroactive species onto electrode surfaces[21,22]. In this technique, a constant current is applied to a micropipette containing a solution of catecholamine. At pH 7.4 the catecholamines are cations because the amine is protonated. When current is applied to the micropipette containing catecholamine, the catecholamine is transported into the bulk solution by electromigration. This technique of discharging molecules from a micropipette is preferable to pressure ejection because less convection occurs.

With amperometry, electroactive species are oxidized when they contact the electrode surface. In contrast, with cyclic voltammetry there is a rest time between application of successive triangle waves to allow the diffusion layer to be replenished. In this interval adsorption can occur. Adsorption of catecholamines at carbon fiber electrodes has been documented and results in a larger signal amplitude than would be predicted for diffusion control[1]. Furthermore, the scan to relatively high potentials used in these studies results in oxide formation on the electrode surface[23] that may cause additional adsorption. To test the effect of adsorption on the temporal response, iontophoresis of catecholamines with amperometry and cyclic voltammetry was compared. The response during epinephrine ejection onto an electrode for 33.3 ms is shown in figure 2A for cyclic voltammetry and compared to amperometry (figure 2B). The similarity in the shapes of these two traces indicates that the temporal response with cyclic voltammetry is similar to amperometry. The full width at half height of the trace measured by cyclic voltammetry is 12% wider than the one measured by amperometry. This percentage difference in half widths decreases as the duration of epinephrine ejection increases. Neither response is instantaneous because of dilution which may occur in the tip of the microinjection pipette. In addition, the applied current pulse has a finite rise time because of the limitations of the electronic components in the constant current device.

To obtain a better estimate of the response time of the carbon-fiber microelectrode, a collector-generator experiment[24] was performed in a solution of 100 μM 3,4-dihydroxybenzylamine (DHBA). The two electrodes were positioned 1 μm apart as shown in figure 3. The potential of the generator electrode (6 μm radius) was stepped from 0 to 900 mV to oxidize DHBA to o-quinone. The flame-etched collector electrode (3 μm radius), was held at -200 mV, a potential sufficient to reduce the generated o-quinone. With a 500 μs potential pulse applied to the generator electrode (figure 3A), the collector current (figure 3C) had a full-width at half height of 594 μs and a half-rise time of 254 μs. With a 10 ms

potential pulse applied to the generator electrode (figure 3B), the collector current reached steady state (figure 3D) with a full-width at half height of 10.68 ms and a half-rise time of 960 μs. In both experiments, the measured full-width at half height is longer than the duration of the applied pulse. The additional time can be attributed to diffusional dispersion of the generated species in solution before it can be detected by the collector electrode[13]. Because the flame-etched electrodes and the glass-encased electrodes were constructed from the same type of carbon fiber and both electrodes were polished before use, their response times should be very similar. These data demonstrate that carbon-fiber microelectrodes have the capability of measuring millisecond time-scale events such as those that occur during exocytosis.

Cyclic Voltammograms during Iontophoresis

Cyclic voltammograms recorded with iontophoresis were used to monitor differences in the time course of the two oxidation waves. As expected, the current trace for catecholamine oxidation to o-quinone (figure 4A) and for o-quinone reduction back to catecholamine (data not shown) have the same time course. However, the second oxidation (figure 4A) lags the catechol oxidation current-time trace in both the rising and falling portions of the trace, indicating that some other process must occur between catechol oxidation and the second oxidation.

The cyclic voltammograms recorded during the rising portion of the current trace in figure 4A do not exhibit a second oxidation wave (figure 4B). About 50 ms later, this wave appears in the cyclic voltammogram as seen in figure 4C. The disappearance of the second oxidation wave lags the disappearance of the other oxidative waves as seen in the cyclic voltammogram taken after epinephrine ejection has stopped (figure 4D). It has been proposed that catecholamines adsorb onto platinum electrodes with the aromatic ring oriented parallel to the electrode surface and the positively charged, amine end of the molecule held away from the electrode surface[25]. If this is the case at carbon surfaces, then

catecholamine may first be oxidized to form o-quinone followed by o-quinone adsorption and reorientation so that the amine becomes accessible to the electrode surface.

Detection of Release from Single Cells

Adrenal medullary cells release catecholamines in discrete packets from vesicles that can be time-resolved by electrochemical measurements[6-9]. Exposure of a cell to 60 mM K^+ causes secretion for about 30 seconds. Figure 5 shows responses measured by cyclic voltammetry from two different cells. The upper traces (figure 5A and 5B) were derived from the current from 550 to 650 mV, the potential where the catechol is oxidized. In each case, release is observed as concentration spikes which occur at irregular intervals. The traces in figure 5C and 5D were derived from the same set of voltammograms but from the current from 1400 to 1425 mV where the second wave of epinephrine occurs. Each spike in figure 5A is accompanied by a spike at the more positive potential in figure 5C indicating that the cell is secreting epinephrine. In contrast, no concentration spikes are seen in figure 5D where spikes occur in figure 5B, indicating that the cell is secreting norepinephrine. One background-subtracted cyclic voltammogram is shown for each case. Comparison of these voltammograms with those in figure 1 confirm the identity of the catecholamine. Differentiating between epinephrine-containing cells and norepinephrine-containing cells at the single cell level may be valuable because recent studies have shown that these two cell types respond differently to various secretion stimuli[26].

Figure 4 shows that when epinephrine is at the electrode surface for very short times ($t < 50$ ms), the second oxidation wave is not observed. Thus it would be difficult to differentiate epinephrine from norepinephrine if the concentration spikes had full-widths of less than 50 ms. With this single-cell data, catecholamine identification was always possible because the full width at half height of 1474 concentration spikes was 70 ms $\pm$ 2 ms (mean $\pm$ s.e.m.).

With amperometry, the integral of a current spike from a single cell gives, by

Faraday's law, the number of catecholamine molecules which reach the electrode surface. This number has been used to estimate the contents of individual vesicles[13]. In cyclic voltammetry, typically the oxidized species is reduced back to the starting species causing some molecules to be oxidized multiple times and thus prohibiting an estimation of the contents of an individual vesicle. Because the waveform employed here does not entail reducing o-quinone back to catecholamine, the charge that is measured is a true measure of the number of molecules of catecholamine detected for a vesicle. The charge measured under 1474 spikes was 1.68 pC ± 0.09 pC (mean ± s.e.m.). This is similar to values that have been obtained by amperometry[14]. This charge corresponds to $5.2 \times 10^{+6}$ molecules of catecholamine detected per vesicle.

Cells with Mixtures of Catecholamines

Release from 75 cells from both the norepinephrine-rich and epinephrine-rich fractions were analyzed at the single cell level for catecholamine type. Cells were categorized based on measurement of release of epinephrine (E), norepinephrine (NE), or a mixture of the two catecholamines. This data is summarized in table 1. A total of thirteen of these cells released mixtures of the two catecholamines. This group of cells is the most biologically interesting because they deviate from traditional wisdom. It is generally believed that each individual bovine adrenal medullary cell releases either epinephrine or norepinephrine but not both. This is because the enzyme phenylethanolamine N-methyltransferase (PNMT), which converts norepinephrine into epinephrine, is found in only some adrenal medullary cells[27]. Cells which contain this enzyme in their cytoplasm release all or mostly epinephrine while those that do not contain the enzyme release only norepinephrine. In practice, several researchers[3,28] have measured the presence of both epinephrine and norepinephrine from the same single cell, implying that the traditional model is incorrect.

Figure 6 shows a series of concentration spikes measured at a single adrenal

medullary cell. The top concentration-time trace shows spikes that were averaged between 500 mV and 600 mV where both epinephrine and norepinephrine are oxidized. Each spike indicates the release of either epinephrine or norepinephrine from the cell. The bottom concentration-time trace in figure 6 shows the corresponding data set averaged between 1300 mV and 1400 mV where the second oxidation occurs for epinephrine but not norepinephrine. The two concentration spikes seen at this high voltage indicate that the two spikes they match up with in the top trace mark epinephrine release while the res spikes in the top trace mark norepinephrine release. Typical cyclic voltammograms measured under an epinephrine concentration spike and under a norepinephrine concentration spike are also shown. While there is no definitive way to prove that small quantities of norepinephrine release do not contribute to the epinephrine spikes, the relative sizes of the two oxidation peaks in the cyclic voltammograms indicate that the spikes designated as epinephrine spikes correspond to release of vesicles that contain primarily epinephrine.

CONCLUSIONS

Epinephrine and norepinephrine can be differentiated using background-subtracted fast-scan cyclic voltammetry at 800 V/s by scanning to sufficiently high voltages to observe a second oxidation wave for epinephrine. A second wave for norepinephrine is not found before the background current becomes very large at the carbon electrode. Temporal response of this technique has been analyzed on a millisecond time-scale by iontophoretic ejection of epinephrine onto the carbon fiber electrode. The data suggest that o-quinone must be adsorbed onto the electrode surface in order for the second oxidation to be observed. For this reason, the second oxidation is optimized when the potential waveform does not involve reduction of o-quinone back to catecholamine. Although this technique necessitates potential scans to relatively high voltages, adsorption does not significantly distort measurements taken on a millisecond time scale when compared to similar measurements taken with constant potential amperometry.

The selectivity of this cyclic voltammetric technique can be used to monitor epinephrine and norepinephrine release from single adrenal medullary cells. Data collected at a rate of every 16.7 ms is sufficiently rapid to resolve release from individual vesicles at a single cell. While most adrenal medullary cells release either epinephrine or norepinephrine exclusively, a small fraction of cells release both catecholamines. This new cyclic voltammetric method can reveal these cells with millisecond resolution.

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Table 1. Chronological Cyclic Voltammetric Identification of Released Catecholamines from Single Adrenal Chromaffin Cells

	Epinephrine-enriched fraction			Norepinephrine-enriched fraction		
	number of E cells	number of mixed cells	number of NE cells	number of E cells	number of mixed cells	number of NE cells
day 1	7	1	2	0	0	5
day 2	11	7	5	0	1	3
day 3	6	1	3	4	0	1
day 4	3	1	2	1	0	1
day 5	0	0	0	1	2	7
totals	27	10	12	6	3	17

Scheme 1. Proposed catecholamine electrochemical reactions observed at carbon-fiber microelectrodes.

Figure 1. Background-subtracted cyclic voltammograms at 800 V/s. (A),(C),(E) 20 μM epinephrine. (B),(D),(E) 20 μM norepinephrine. (A),(B) The potential scan limits are -450 and +950 mV. (C),(D) The potential scan limits are -450 and +1425 mV. (E),(F) The potential scan limits are +100 and +1425 mV.

Figure 2. Electrochemical responses during iontophoretic ejection of epinephrine at a carbon-fiber electrode for 33.3 ms. (A) Measured by cyclic voltammetry scanning from +100 to +1425 mV at 800 V/s every 16.7 ms. Each circle represents a data point taken during a single scan of the electrode. Data for this trace was averaged between +500 and +600 mV. (B) Measured by amperometry with the electrode held at +650 mV. Each circle represents the time at which data was sampled.

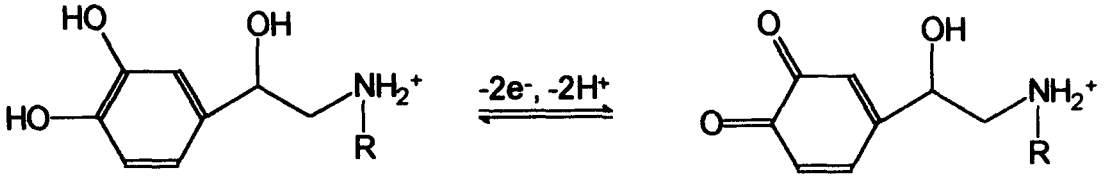
Figure 3. Currents measured at a collector electrode after the generator electrode was pulsed from 0 to +900 mV in 100 μM DHBA. The collector electrode was held at -200 mV. Collector and generator electrode placement are shown in the sketch. (A) Generator electrode 500 μs potential pulse. (B) Generator electrode 10 ms potential pulse. (C) Collector electrode current response to potential pulse in (A). This current was analog low-pass filtered at 5 kHz and also digitally low-pass filtered at 1 kHz. (D) Collector electrode current response to potential pulse in (B). This current was analog low pass filtered at 1 kHz.

Figure 4. (A) Current-time trace from an iontophoretic ejection of epinephrine onto the electrode for 167 ms. Squares represent data averaged between 500 and 600 mV. Circles

represent data measured between 1400 and 1425 mV. Each symbol represents the measurements taken during one cycle of the electrode. (B) Cyclic voltammogram of (A) averaged between 16.7 and 50 ms after the start of ejection as indicated by the rectangle. No second oxidation wave appears in the voltammogram. (C) Voltammogram of (A) averaged between 133 and 167 ms after the start of ejection. (D) Voltammogram of (A) averaged between 16.7 and 50 ms after ejection of epinephrine ceased.

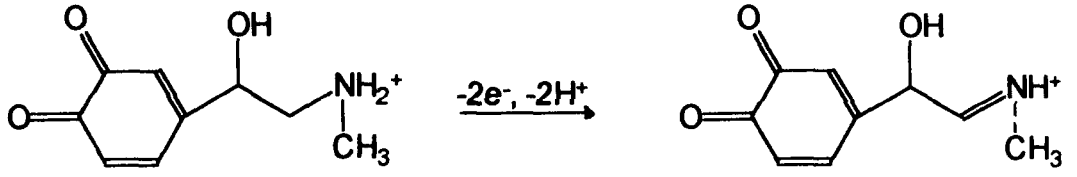
Figure 5. (A) Concentration-time trace measured at a single cell that released only epinephrine. This trace is of data averaged between 550 and 650 mV. The arrow indicates the time that potassium was ejected at the cell to stimulate catecholamine release. A cyclic voltammogram measured under one of the spikes confirms that the measured substance is epinephrine. (B) Analogous data collected at a cell that released only norepinephrine. (C) Concentration-time trace from the same data set as in (A) averaged between 1400 and 1425 mV. Spikes at the higher potential are seen only when epinephrine is released from the cell. (D) Current-time trace from the same data set as in (B) averaged between 1400 and 1425 mV. No spikes are seen at this potential because only norepinephrine is released from the cell.

Figure 6. The top concentration-time trace shows catecholamine release from a single cell where data was averaged between 500 and 600 mV. The bottom trace is the corresponding trace averaged between 1400 and 1425 mV. The two spikes observed in the bottom trace mark epinephrine release. Voltammograms measured under an epinephrine spike and a norepinephrine spike are also shown.



(1)

$E_{p,a} \approx 460$ mV



(2)

$E_p \approx 1425$ mV

For epinephrine, R=CH_3. For norepinephrine, R=H.

Scheme 1

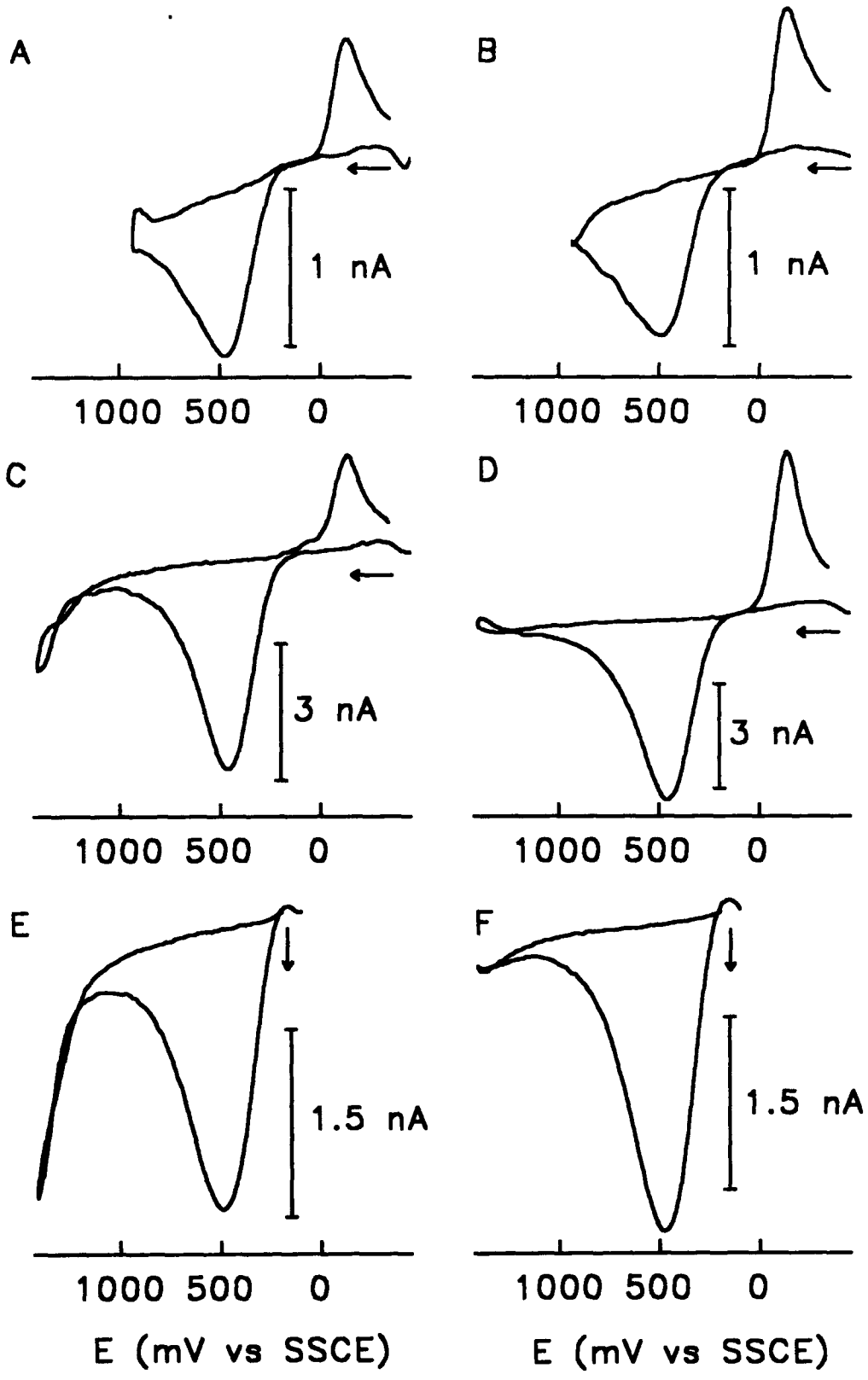

A
1 nA
1000 500 0
B
1 nA
1000 500 0
C
3 nA
1000 500 0
D
3 nA
1000 500 0
E
1.5 nA
1000 500 0
E (mV vs SSCE)
F
1.5 nA
1000 500 0
E (mV vs SSCE)

Figure 1

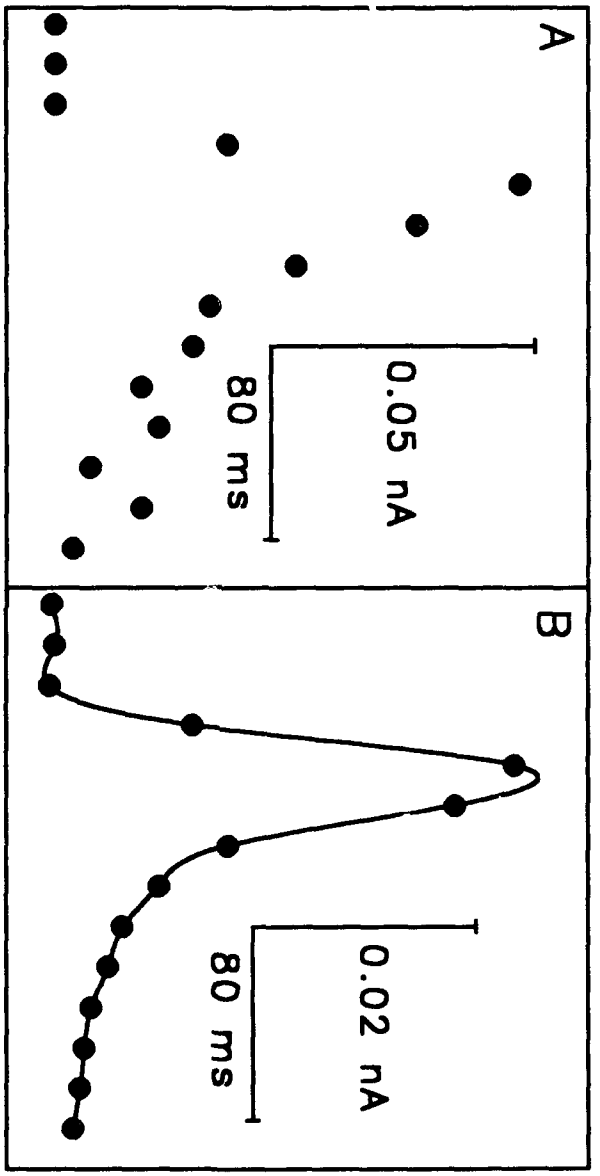


Figure 2

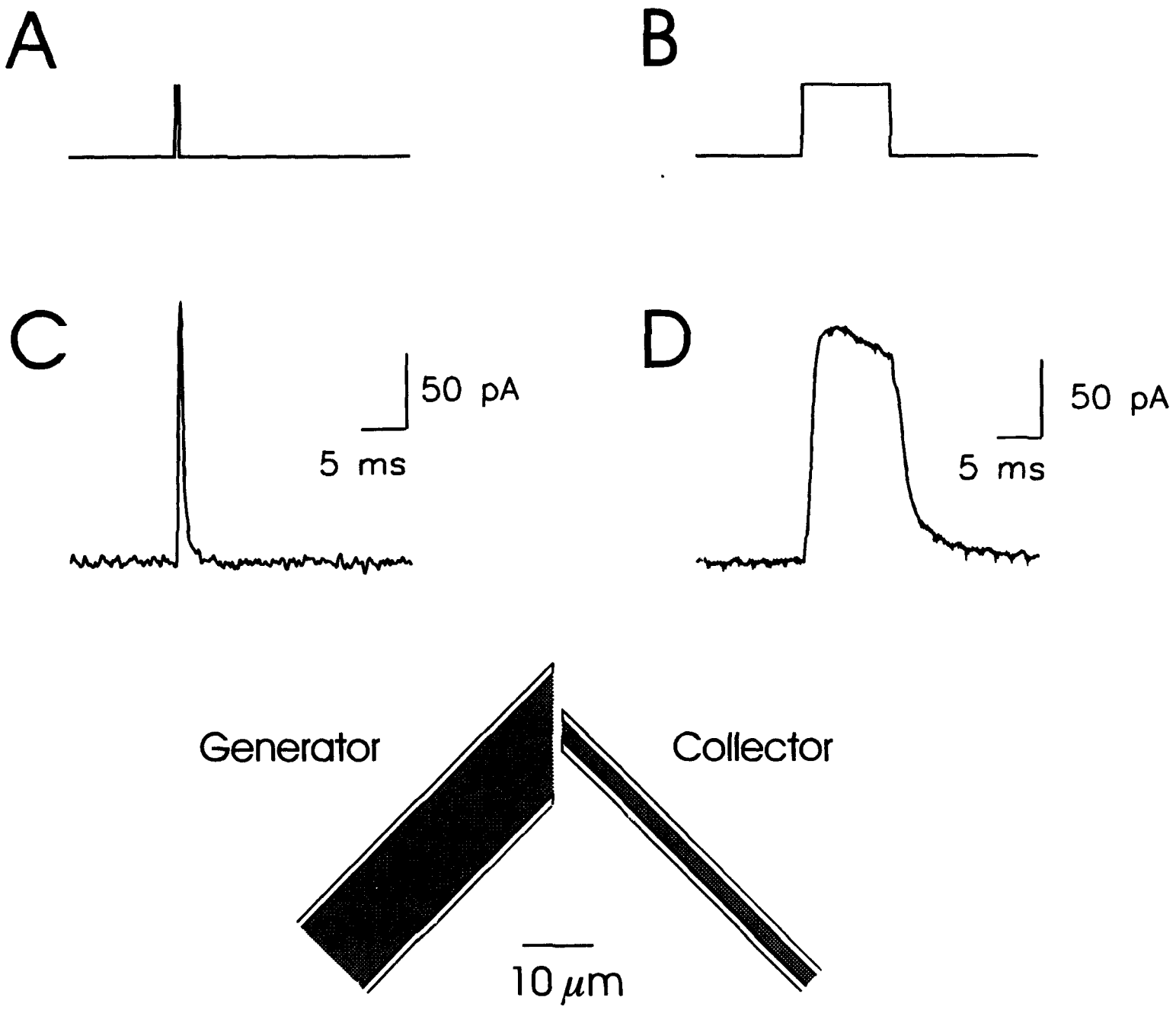


Figure 3

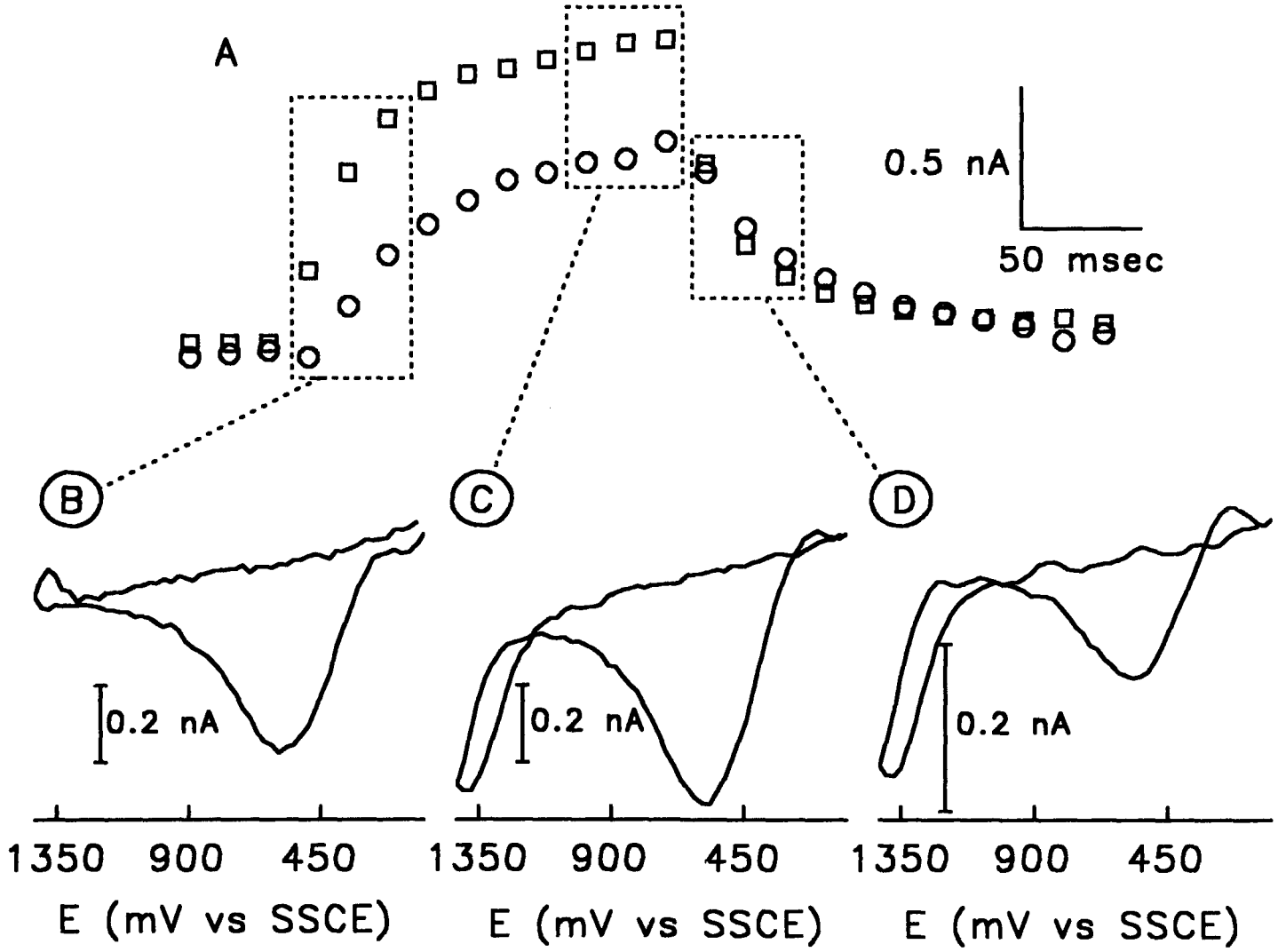

A
0.5 nA
50 msec
B
C
D
0.2 nA
0.2 nA
0.2 nA
1350 900 450
1350 900 450
1350 900 450
E (mV vs SSCE)
E (mV vs SSCE)
E (mV vs SSCE)

Figure 4

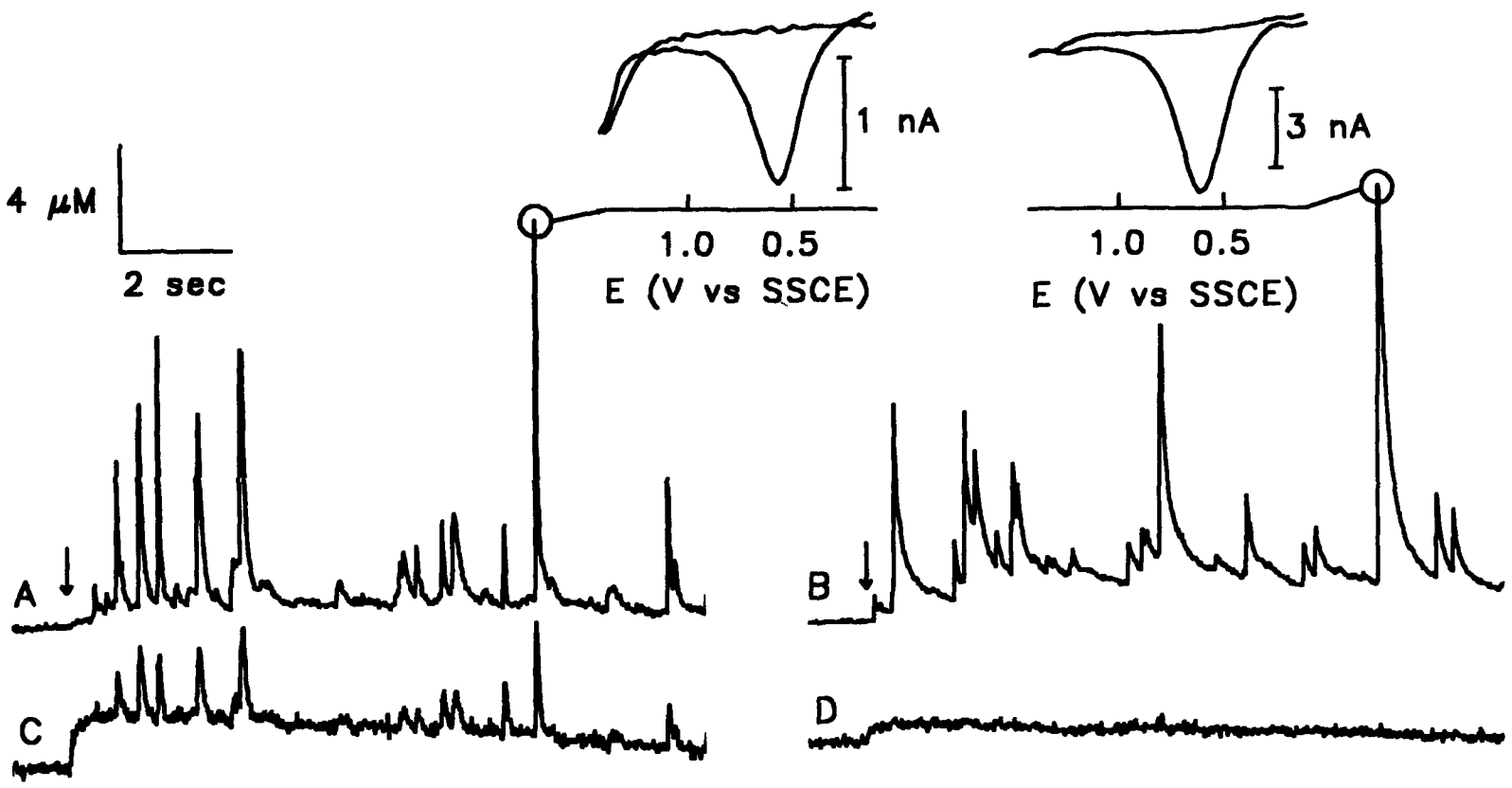


Figure 5

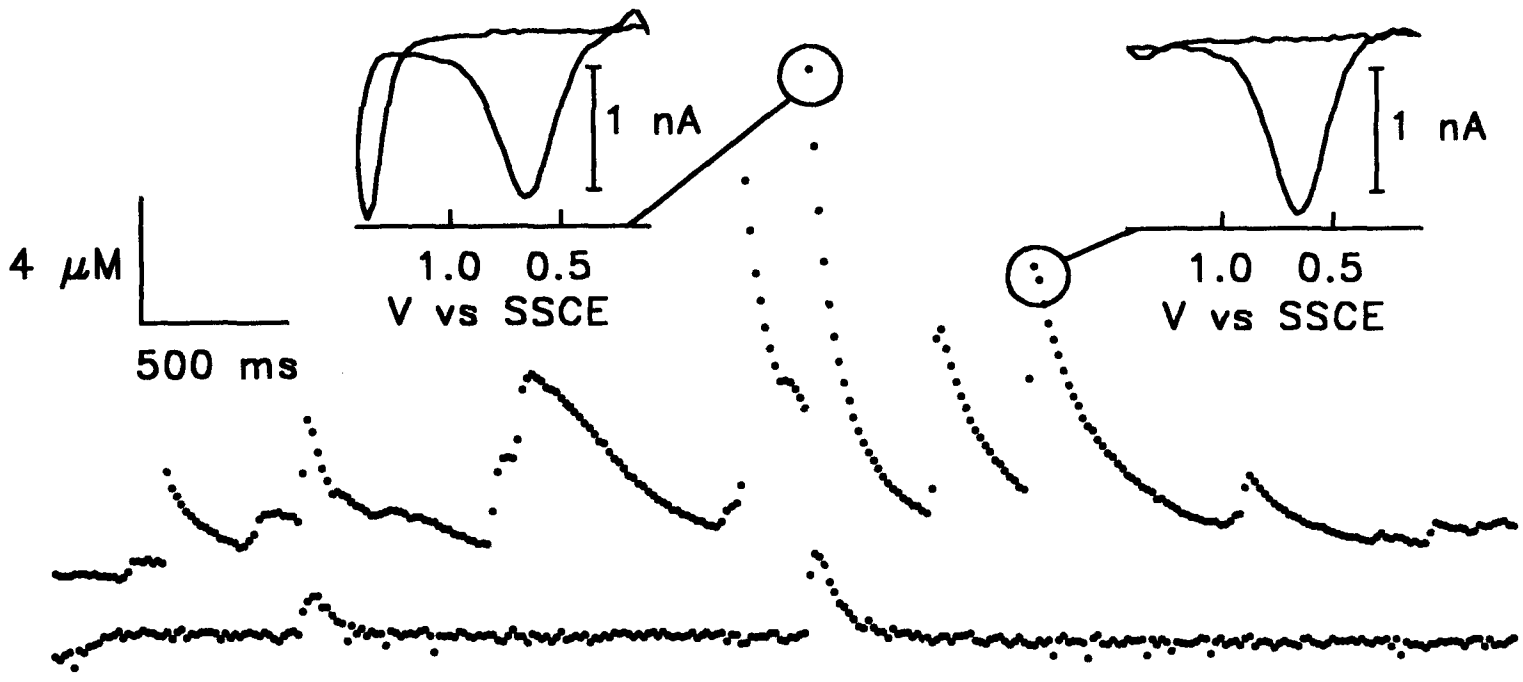


Figure 6